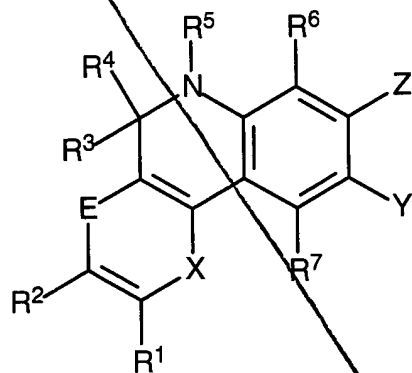


We claim

1. A compound of the formula



wherein

- 10 R¹ and R² are independently H, cyano, halogen, carboxylic acid, or sulfonic acid; or a C₁-C₆ alkyl or alkoxy that is optionally substituted by carboxylic acid, sulfonic acid, or halogen; or an aryl or heteroaryl ring that is optionally substituted one or more times by C₁-C₆ alkyl, C₁-C₆ perfluoroalkyl, cyano, halogen, azido, carboxylic acid, sulfonic acid, or halomethyl; or -L-R_x; or -L-S_c;
- 15 or R¹ in combination with R² forms a fused aromatic or heteroaromatic ring that is optionally sulfonated one or more times, or which ring is substituted by -L-R_x or -L-S_c;
- or R² in combination with R³ forms a 5- or 6-membered alicyclic ring;
- 20 R³ and R⁴ are independently H, C₁-C₆ alkyl that is optionally substituted by carboxylic acid, sulfonic acid, amino, hydroxy, or halogen; or an aromatic or heteroaromatic ring that is optionally substituted one or more times by C₁-C₆ alkyl, C₁-C₆ alkoxy, C₁-C₆ perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl; or -L-R_x; or -L-S_c;
- 25 or R³ in combination with R⁴ forms a 5- or 6-membered alicyclic ring;

9/2/13
R⁵ is H, methyl, carboxymethyl, a C₂-C₆ alkyl that is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen; or R⁵ is an aryl or heteroaryl ring that is optionally substituted one or more times by C₁-C₆ alkyl, C₁-C₆ perfluoroalkyl, cyano, halogen,
5 carboxylic acid, sulfonic acid, or halomethyl; or -L-R_x; or -L-S_c;

R⁶ is H, cyano, halogen, carboxylic acid, or sulfonic acid; or a C₁-C₆ alkyl or alkoxy that is optionally substituted by carboxylic acid, sulfonic acid, or halogen; or an aryl or heteroaryl ring that is optionally substituted one or more times by C₁-C₆ alkyl, C₁-C₆ perfluoroalkyl, cyano, halogen, azido, carboxylic acid, sulfonic acid, or halomethyl; or -L-R_x; or -L-S_c;

or R⁴ in combination with R⁵, or R⁵ in combination with R⁶, forms a 5- or 6-membered alicyclic ring;

15 R⁷ is hydrogen, alkyl having 1-6 carbons, or alkoxy having 1-6 carbons; or -L-R_x; or -L-S_c;

one of X and E is O, S, NR⁸, or CR^{1'}=CR^{2'}, and the other is absent;

20 wherein R⁸ is H, methyl, carboxymethyl, or a C₂-C₆ alkyl that is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen; or -L-R_x; or -L-S_c;
and

25 R^{1'} and R^{2'} are independently H, cyano, halogen, carboxylic acid, or sulfonic acid; or a C₁-C₆ alkyl or alkoxy that is optionally substituted by carboxylic acid, sulfonic acid, or halogen; or an aryl or heteroaryl ring that is optionally substituted one or more times by C₁-C₆ alkyl, C₁-C₆ perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl; or -L-R_x; or -L-S_c;

30 Y is H, OH, NH₂, NO, or -(CO)-R⁹, or -(CO)-O-R¹⁰, where R⁹ and R¹⁰ are H, C₁-C₆ alkyl, or a substituted or unsubstituted aryl or heteroaryl ring system having 1-2 rings;

Z is H, OH, NHR^{17} , SH, or $\text{C}(\text{CR}^{11}\text{R}^{12})_2\text{OH}$; where R^{17} is a $\text{C}_1\text{-C}_6$ alkyl that is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen; R^{11} and R^{12} are independently $\text{C}_1\text{-C}_6$ alkyls that are optionally substituted by carboxylic acid, sulfonic acid, or halogen, or R^{11} and R^{12} taken in combination form a 5- or 6-membered alicyclic ring;

5

wherein L is a covalent linkage;

R_x is a reactive group; and

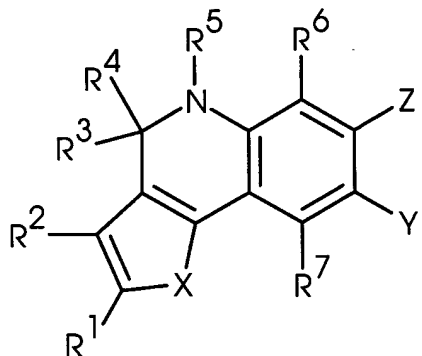
10

S_c is a conjugated substance.

2. A compound, as claimed in Claim 1, wherein one of X and E is O, S, or $\text{CR}^{1'}=\text{CR}^{2'}$, and the other is absent.

15

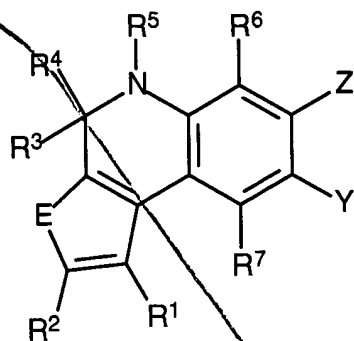
3. A compound, as claimed in Claim 1, having the formula



wherein X is O or S.

20

4. A compound, as claimed in Claim 1, having the formula



wherein E is O or S.

5. A compound, as claimed in Claim 2, wherein X is S.

6. A compound as claimed in Claim 1, wherein

R¹ is H or sulfonic acid;

R³ and R⁴ are each methyl;

R⁶ and R⁷ are each hydrogen or methyl; and

Z is OH.

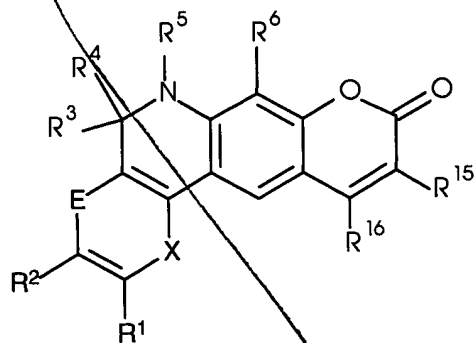
7. A compound, as claimed in Claim 1, wherein Y is H or -(CO)-H or NO.

8. A compound, as claimed in Claim 1, wherein each L is independently a single covalent bond, or L is a covalent linkage having 1-24 nonhydrogen atoms selected from the group consisting of C, N, O, P, and S and is composed of any combination of single, double, triple or aromatic carbon-carbon bonds, carbon-nitrogen bonds, nitrogen-nitrogen bonds, carbon-oxygen bonds, carbon-sulfur bonds, phosphorus-oxygen bonds, and phosphorus-nitrogen bonds.

5 9. A compound, as claimed in Claim 1, wherein R_x is an acrylamide, an activated ester of a carboxylic acid, an acyl azide, an acyl nitrile, an aldehyde, an alkyl halide, an amine, an anhydride, an aniline, an aryl halide, an azide, an aziridine, a boronate, a carboxylic acid, a diazoalkane, a haloacetamide, a halotriazine, a hydrazine, an imido ester, an isocyanate, an isothiocyanate, a maleimide, a phosphoramidite, a reactive platinum complex, a sulfonyl halide, or a thiol group.

10 10. A compound, as claimed in Claim 1, wherein S_c is an amino acid, a peptide, a protein, a tyramine, a monosaccharide, a polysaccharide, an ion-complexing moiety, a nucleoside, a nucleotide, an oligonucleotide, a nucleic acid, a hapten, a psoralen, a drug, a hormone, a lipid, a lipid assembly, a polymer, a polymeric microparticle, a biological cell, or a virus.

11. A compound of the formula



5 R^1 , R^2 , and R^6 are independently H, cyano, halogen, carboxylic acid, or sulfonic acid; or a C_1 - C_6 alkyl or alkoxy that is optionally substituted by carboxylic acid, sulfonic acid, or halogen; or an aromatic or heteroaromatic ring that is optionally substituted one or more times by C_1 - C_6 alkyl, C_1 - C_6 perfluoroalkyl, cyano, halogen, azido, carboxylic acid, sulfonic acid, or halomethyl; or $-L-R_x$; or $-L-S_C$;

10

or R^1 in combination with R^2 forms a fused aromatic or heteroaromatic ring that is optionally sulfonated one or more times;

15

R^3 and R^4 are independently H, C_1 - C_6 alkyl that is optionally substituted by carboxylic acid, sulfonic acid, amino, hydroxy, or halogen; or an aromatic or heteroaromatic ring that is optionally substituted one or more times by C_1 - C_6 alkyl, C_1 - C_6 alkoxy, C_1 - C_6 perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl; or $-L-R_x$; or $-L-S_C$;

20

or R^2 in combination with R^3 , or R^3 in combination with R^4 , forms a 5- or 6-membered alicyclic ring;

25

R^5 is H, methyl, carboxymethyl, a C_2 - C_6 alkyl that is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen; or R^5 is an aryl or heteroaryl ring that is optionally substituted one or more times by C_1 - C_6 alkyl, C_1 - C_6 perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl; or $-L-R_x$; or $-L-S_C$;

or R⁴ in combination with R⁵, or R⁵ in combination with R⁶, forms a 5- or 6-membered alicyclic ring;

5 one of X and E is O, S, NR⁸, or CR^{1'}=CR^{2'}; the other is absent;

wherein R⁸ is H, methyl, carboxymethyl, or a C₂-C₆ alkyl that is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen; or -L-R_x; or -L-S_C;
and

10 R^{1'} and R^{2'} are independently H, cyano, halogen, carboxylic acid, or sulfonic acid; or a C₁-C₆ alkyl or alkoxy that is optionally substituted by carboxylic acid, sulfonic acid, or halogen; or an aryl or heteroaryl ring that is optionally substituted one or more times by C₁-C₆ alkyl, C₁-C₆ perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic
15 acid, or halomethyl; or -L-R_x; or -L-S_C;

R¹⁵ and R¹⁶ are hydrogen, cyano, nitro, halogen, carboxylic acid, or sulfonic acid; or a C₁-C₆ alkyl that is optionally substituted by carboxylic acid, sulfonic acid, or halogen; or an aromatic or heteroaromatic ring system having 1-2 fused rings that is optionally
20 substituted one or more times by C₁-C₆ alkyl, C₁-C₆ perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl; or -L-R_x; or -L-S_C;

wherein L is a covalent linkage;

25 R_x is a reactive group; and

S_C is a conjugated substance.

12. A compound, as claimed in Claim 11, wherein one of X and E is O or S.

30

13. A compound, as claimed in Claim 12, wherein

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com.
5 R^6 and R^7 are H;

R^3 and R^4 are each methyl;

R^1 is H or sulfonic acid;

one of R^{15} and R^{16} is $-L-R_x$ or $-L-S_c$, and the other is hydrogen, C_1-C_6 alkyl, C_1-C_6 perfluoroalkyl; or cyano;

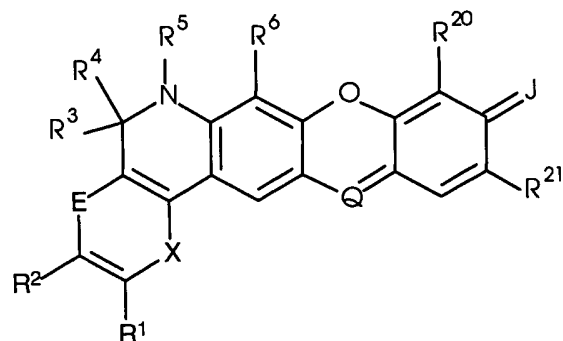
10 wherein L is a single covalent bond, or L is a covalent linkage having 1-24 nonhydrogen atoms selected from the group consisting of C, N, O, P, and S and is composed of any combination of single, double, triple or aromatic carbon-carbon bonds, carbon-nitrogen bonds, nitrogen-nitrogen bonds, carbon-oxygen bonds, carbon-sulfur bonds, phosphorus-oxygen bonds, and phosphorus-nitrogen bonds, and

15 wherein R_x , when present, is an acrylamide, an activated ester of a carboxylic acid, an acyl azide, an acyl nitrile, an aldehyde, an alkyl halide, an amine, an anhydride, an aniline, an aryl halide, an azide, an aziridine, a boronate, a carboxylic acid, a diazoalkane, a haloacetamide, a halotriazine, a hydrazine, an imido ester, an isocyanate, an isothiocyanate, a maleimide, a phosphoramidite, a reactive platinum complex, a sulfonyl
20 halide, or a thiol group; and

wherein S_c , when present, is an amino acid, a peptide, a protein, a tyramine, a monosaccharide, a polysaccharide, an ion-complexing moiety, a nucleoside, a nucleotide, an oligonucleotide, a nucleic acid, a hapten, a psoralen, a drug, a hormone, a lipid, a lipid assembly, a polymer, a polymeric microparticle, a biological cell, or a virus.

25 14. A compound, as claimed in Claim 11, wherein one of R^{15} and R^{16} is an aromatic or heteroaromatic ring system having 1-2 fused rings that is optionally substituted one or more times by C_1-C_6 alkyl, C_1-C_6 perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl.
30

15. A compound of the formula



5 wherein

R¹, R², and R⁶ are independently H, cyano, nitro, halogen, carboxylic acid, or sulfonic acid; or a C₁-C₆ alkyl or alkoxy that is optionally substituted by carboxylic acid, sulfonic acid, or halogen; or an aromatic or heteroaromatic ring that is optionally substituted one or more
10 times by C₁-C₆ alkyl, C₁-C₆ perfluoroalkyl, cyano, halogen, azido, carboxylic acid, sulfonic acid, or halomethyl; or -L-R_x; or -L-S_C;

or R¹ in combination with R² forms a fused aromatic or heteroaromatic ring that is optionally sulfonated one or more times;

15

R³ and R⁴ are independently H, C₁-C₆ alkyl that is optionally substituted by carboxylic acid, sulfonic acid, amino, hydroxy, or halogen; or an aromatic or heteroaromatic ring that is optionally substituted one or more times by C₁-C₆ alkyl, C₁-C₆ perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl; or -L-R_x; or -L-S_C;

20

or R² in combination with R³, or R³ in combination with R⁴, forms a 5- or 6-membered alicyclic ring;

25

R⁵ is H, methyl, carboxymethyl, a C₂-C₆ alkyl that is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen; or R⁵ is an aryl or heteroaryl ring that is optionally

substituted one or more times by C_1-C_6 alkyl, C_1-C_6 perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl; or R^5 is $-L-R_x$ or $-L-S_C$;

5 or R^4 in combination with R^5 , or R^5 in combination with R^6 , forms a 5- or 6-membered alicyclic ring;

one of X and E is O, S, NR^8 , or $CR^{1'}=CR^{2'}$; and the other is absent;

10 wherein R^8 is H, methyl, carboxymethyl, or a C_2-C_6 alkyl that is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen; or $-L-R_x$; or $-L-S_C$; and

15 $R^{1'}$ and $R^{2'}$ are independently H, cyano, halogen, carboxylic acid, or sulfonic acid; or a C_1-C_6 alkyl or alkoxy that is optionally substituted by carboxylic acid, sulfonic acid, or halogen; or an aryl or heteroaryl ring that is optionally substituted one or more times by C_1-C_6 alkyl, C_1-C_6 perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl; or $-L-R_x$; or $-L-S_C$;

20 R^{20} and R^{21} are hydrogen, cyano, halogen, carboxylic acid, or sulfonic acid; or a C_1-C_6 alkyl or alkoxy that is optionally substituted by carboxylic acid, sulfonic acid, or halogen; or an aromatic or heteroaromatic ring that is optionally substituted one or more times by C_1-C_6 alkyl, C_1-C_6 perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl; or $-L-R_x$; or $-L-S_C$;

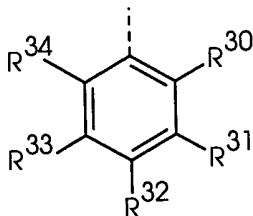
25 J is O or $NR^{37}R^{38}$;

30 where R^{37} and R^{38} are independently H, C_1-C_6 alkyl that is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen; an aryl or heteroaryl ring; or R^{37} in combination with R^{38} forms a saturated 5- or 6-membered heterocycle that is a piperidine, a morpholine, a pyrrolidine or a piperazine, each of which is optionally substituted by methyl, carboxylic acid, or a carboxylic acid ester of a C_1-C_6 alkyl; or

-L-R_x or -L-S_C;

or R³⁷ in combination with R²⁰, or R³⁸ in combination with R²¹, or both, form a 5- or 6-membered ring that is saturated or unsaturated, and is optionally substituted by one or more sulfonic acids, or C₁-C₆ alkyl that is optionally substituted by sulfonic acid;

Q is N or CR²⁸, wherein R²⁸ is H, F, CN, carboxylic acid, or a carboxylic acid ester of a C₁-C₆ alcohol; or R²⁸ is a C₁-C₆ alkyl that is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen; or R²⁸ has the formula



where R³⁰, R³¹, R³², R³³ and R³⁴ are independently H, F, Cl, Br, I, sulfonic acid, carboxylic acid, CN, nitro, hydroxy, azido, amino, hydrazino; or C₁-C₁₈ alkyl, C₁-C₁₈ alkoxy, C₁-C₁₈ alkylthio, C₁-C₁₈ alkanoylamino, C₁-C₁₈ alkylaminocarbonyl, C₂-C₃₆ dialkylaminocarbonyl, C₁-C₁₈ alkyloxycarbonyl, or C₆-C₁₈ arylcarboxamido, the alkyl or aryl portions of which are optionally substituted one or more times by F, Cl, Br, I, hydroxy, carboxylic acid, a carboxylic acid ester of a C₁-C₆ alcohol, sulfonic acid, amino, alkylamino, dialkylamino or alkoxy, the alkyl portions of each having 1-6 carbons; or one pair of adjacent substituents R³¹ and R³², R³² and R³³ or R³³ and R³⁴, when taken in combination, form a fused 6-membered aromatic ring that is optionally further substituted by carboxylic acid; or one or more of R³⁰, R³¹, R³², R³³ and R³⁴ is -L-R_x or -L-S_C; and

wherein L is a covalent linkage;

R_x is a reactive group; and

S_c is a conjugated substance.

16. A compound, as claimed in Claim 15, wherein Q is N.

17. A compound, as claimed in Claim 15, wherein J is O and Q is CR^{28} .

18. A compound, as claimed in Claim 17, wherein one of R^5 , R^{21} , R^{30} , R^{31} , R^{32} , R^{33} , and R^{34} is $-L-R_x$ or $-L-S_c$.

19. A compound, as claimed in Claim 15, wherein

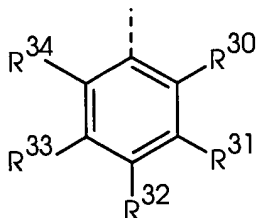
R^3 and R^4 are each methyl;

R^1 is H or a sulfonic acid;

R^6 is H; and

J is $NR^{37}R^{38}$.

20. A compound, as claimed in Claim 19, wherein Q has the formula CR^{28} , wherein R^{28} has the formula



wherein one of R^{30} - R^{34} is $-L-R_x$ or $-L-S_c$; and

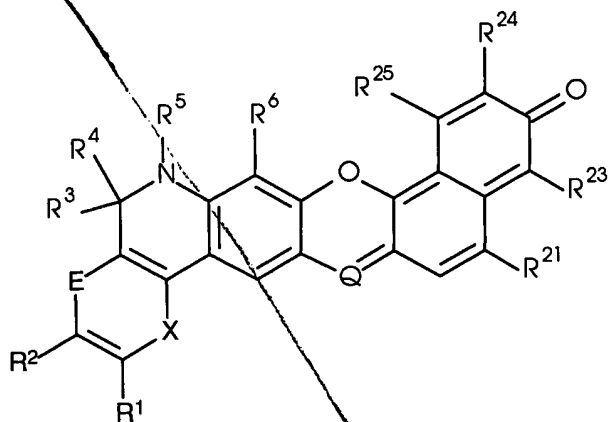
wherein L is a single covalent bond, or L is a covalent linkage having 1-24 nonhydrogen

atoms selected from the group consisting of C, N, O, P, and S and is composed of any combination of single, double, triple or aromatic carbon-carbon bonds, carbon-nitrogen bonds, nitrogen-nitrogen bonds, carbon-oxygen bonds, carbon-sulfur bonds, phosphorus-oxygen bonds, and phosphorus-nitrogen bonds, and

5 wherein R_x , when present, is an acrylamide, an activated ester of a carboxylic acid, an acyl azide, an acyl nitrile, an aldehyde, an alkyl halide, an amine, an anhydride, an aniline, an aryl halide, an azide, an aziridine, a boronate, a carboxylic acid, a diazoalkane, a haloacetamide, a halotriazine, a hydrazine, an imido ester, an isocyanate, an isothiocyanate, a maleimide, a phosphoramidite, a reactive platinum complex, a sulfonyl
10 halide, or a thiol group; and

wherein S_c , when present, is an amino acid, a peptide, a protein, a tyramine, a monosaccharide, a polysaccharide, an ion-complexing moiety, a nucleoside, a nucleotide, an oligonucleotide, a nucleic acid, a hapten, a psoralen, a drug, a hormone, a lipid, a lipid assembly, a polymer, a polymeric microparticle, a biological cell, or a virus.

21. A compound of the formula



5 wherein

R^1 , R^2 , and R^6 are independently H, cyano, halogen, carboxylic acid, or sulfonic acid; or a C_1 - C_6 alkyl or alkoxy that is optionally substituted by carboxylic acid, sulfonic acid, or halogen; or an aromatic or heteroaromatic ring that is optionally substituted one or more times by C_1 - C_6 alkyl, C_1 - C_6 perfluoroalkyl, cyano, halogen, azido, carboxylic acid, sulfonic acid, or halomethyl; or $-L-R_x$; or $-L-S_c$;

or R^1 in combination with R^2 forms a fused aromatic or heteroaromatic ring that is optionally sulfonated one or more times;

R^3 and R^4 are independently C_1 - C_6 alkyl that is optionally substituted by carboxylic acid, sulfonic acid, amino, hydroxy, or halogen; or an aromatic or heteroaromatic ring that is optionally substituted one or more times by C_1 - C_6 alkyl, C_1 - C_6 perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl;

or R^2 in combination with R^3 , or R^3 in combination with R^4 , forms a 5- or 6-membered alicyclic ring;

R^5 is H, methyl, carboxymethyl, a C_2-C_6 alkyl that is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen; or R^5 is an aryl or heteroaryl ring that is optionally substituted one or more times by C_1-C_6 alkyl, C_1-C_6 perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl;

or R^4 in combination with R^5 , or R^5 in combination with R^6 , forms a 5- or 6-membered alicyclic ring;

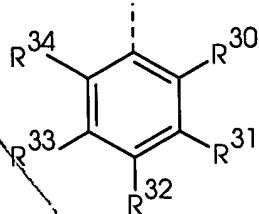
one of E and X is O, S, NR^8 , or $CR^1=CR^2$; the other is absent;

wherein R^8 is H, methyl, carboxymethyl, or a C_2-C_6 alkyl that is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen; and

R^1 and R^2 are independently H, cyano, halogen, carboxylic acid, or sulfonic acid; or a C_1-C_6 alkyl or alkoxy that is optionally substituted by carboxylic acid, sulfonic acid, or halogen; or an aryl or heteroaryl ring that is optionally substituted one or more times by C_1-C_6 alkyl, C_1-C_6 perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl;

R^{21} , R^{23} , R^{24} , and R^{25} are hydrogen, cyano, nitro, halogen, carboxylic acid, or sulfonic acid; or a C_1-C_6 alkyl that is optionally substituted by carboxylic acid, sulfonic acid, or halogen; or an aromatic or heteroaromatic ring that is optionally substituted one or more times by C_1-C_6 alkyl, C_1-C_6 perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl; or $-L-R_x$; or $-L-S_C$;

Q is N or CR^{28} , wherein R^{28} is H, F, CN, carboxylic acid, or a carboxylic acid ester of a C_1-C_6 alcohol; or R^{28} is a C_1-C_6 alkyl that is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen; or R^{28} has the formula



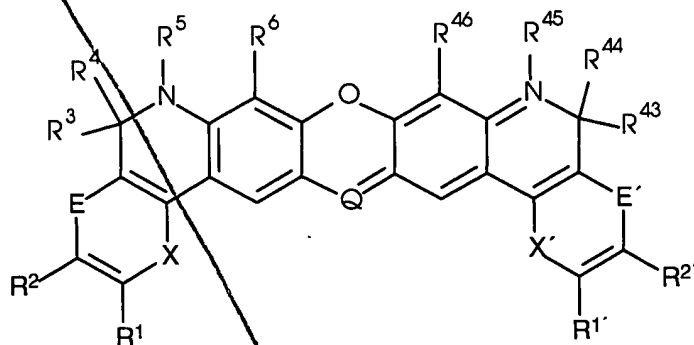
where R^{30} , R^{31} , R^{32} , R^{33} and R^{34} are independently H, F, Cl, Br, I, sulfonic acid, carboxylic acid, CN, nitro, hydroxy, azido, amino, hydrazino; or C_1-C_{18} alkyl, C_1-C_{18} alkoxy, C_1-C_{18} alkylthio, C_1-C_{18} alkanoylamino, C_1-C_{18} alkylaminocarbonyl, C_2-C_{36} dialkylaminocarbonyl, C_1-C_{18} alkyloxycarbonyl, or C_6-C_{18} arylcarboxamido, the alkyl or aryl portions of which are optionally substituted one or more times by F, Cl, Br, I, hydroxy, carboxylic acid, a carboxylic acid ester of a C_1-C_6 alcohol, amino, alkylamino, dialkylamino or alkoxy, the alkyl portions of each having 1-6 carbons; or one pair of adjacent substituents R^{31} and R^{32} , R^{32} and R^{33} or R^{33} and R^{34} , when taken in combination, form a fused 6-membered aromatic ring that is optionally further substituted by carboxylic acid; or one or more of R^{30} , R^{31} , R^{32} , R^{33} and R^{34} is $-L-R_x$ or $-L-S_c$; and

wherein L is a covalent linkage;

R_x is a reactive group; and

S_c is a conjugated substance.

22. A compound having the formula



wherein

R¹, R², R⁶, R⁴¹, R⁴², and R⁴⁶ are independently H, cyano, nitro, halogen, carboxylic acid, or sulfonic acid; or a C₁-C₆ alkyl or alkoxy that is optionally substituted by carboxylic acid, sulfonic acid, or halogen; or an aromatic or heteroaromatic ring that is optionally substituted one or more times by C₁-C₆ alkyl, C₁-C₆ perfluoroalkyl, cyano, halogen, azido, carboxylic acid, sulfonic acid, or halomethyl; or -L-R_x; or -L-S_C;

or R¹ in combination with R², or R⁴¹ in combination with R⁴², or both, forms a fused aromatic or heteroaromatic ring that is optionally sulfonated one or more times;

R³, R⁴, R⁴³, and R⁴⁴ are independently H, C₁-C₆ alkyl that is optionally substituted by carboxylic acid, sulfonic acid, amino, hydroxy, or halogen; or an aromatic or heteroaromatic ring that is optionally substituted one or more times by C₁-C₆ alkyl, C₁-C₆ perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl;

or R² in combination with R³, R⁴² in combination with R⁴³, or R³ in combination with R⁴, or R⁴³ in combination with R⁴⁴, or any combination thereof, forms a 5- or 6-membered alicyclic ring;

R^5 and R^{45} are independently H, methyl, carboxymethyl, a C_2-C_6 alkyl that is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen; or R^5 is an aryl or heteroaryl ring that is optionally substituted one or more times by C_1-C_6 alkyl, C_1-C_6 perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl;

5

or R^4 in combination with R^5 , or R^5 in combination with R^6 , or R^{44} in combination with R^{45} , or R^{45} in combination with R^{46} , or any combination thereof, forms a 5- or 6-membered alicyclic ring;

10 one of E and X is O, S, NR^8 , or $CR^{1'}=CR^{2'}$; the other is absent; and one of E' and X' is O, S, NR^8 , or $CR^{1'}=CR^{2'}$; the other is absent;

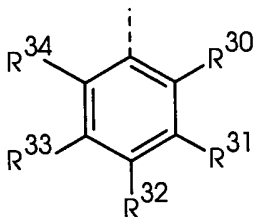
wherein R^8 is H, methyl, carboxymethyl, or a C_2-C_6 alkyl that is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen; and

$R^{1'}$ and $R^{2'}$ are independently H, cyano, halogen, carboxylic acid, or sulfonic acid; or a C_1-C_6 alkyl or alkoxy that is optionally substituted by carboxylic acid, sulfonic acid, or halogen; or an aryl or heteroaryl ring that is optionally substituted one or more times by C_1-C_6 alkyl, C_1-C_6 perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl;

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Q is N or CR^{28} , wherein R^{28} is H, F, CN, carboxylic acid, or a carboxylic acid ester of a C_1-C_6 alcohol; or R^{28} is a C_1-C_6 alkyl that is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen; or R^{28} has the formula

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where R^{30} , R^{31} , R^{32} , R^{33} and R^{34} are independently H, F, Cl, Br, I, sulfonic acid, carboxylic acid, CN, nitro, hydroxy, azido, amino, hydrazino; or C_1-C_{18} alkyl, C_1-C_{18} alkoxy, C_1-C_{18} alkylthio, C_1-C_{18} alkanoylamino, C_1-C_{18} alkylaminocarbonyl, C_2-C_{36} dialkylaminocarbonyl, C_1-C_{18} alkylloxycarbonyl, or C_6-C_{18} arylcarboxamido, the alkyl or aryl portions of which are optionally substituted one or more times by F, Cl, Br, I, hydroxy, carboxylic acid, a carboxylic acid ester of a C_1-C_6 alcohol, amino, alkylamino, dialkylamino or alkoxy, the alkyl portions of each having 1-6 carbons; or one pair of adjacent substituents R^{31} and R^{32} , R^{32} and R^{33} or R^{33} and R^{34} , when taken in combination, form a fused 6-membered aromatic ring that is optionally further substituted by carboxylic acid; or one or more of R^{30} , R^{31} , R^{32} , R^{33} and R^{34} is $-L-R_x$ or $-L-S_c$; and

wherein L is a covalent linkage;

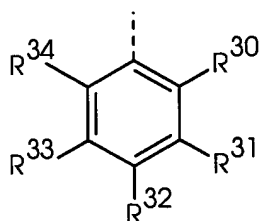
R_x is a reactive group; and

S_c is a conjugated substance.

23. A compound, as claimed in Claim 22, wherein

$X = X'$, $E = E'$, $R^1 = R^{41}$, and $R^2 = R^{42}$.

24. A compound, as claimed in Claim 22, wherein Q has the formula CR^{28} , and R^{28} has the formula



25. A compound, as claimed in Claim 24, wherein one of R^5 , R^{21} , R^{30} , R^{31} , R^{32} , R^{33} , R^{34} , and R^{45} is $-L-R_x$ or $-L-S_c$.

26. A compound, as claimed in Claim 24, wherein

R^3 , R^4 , R^{43} , and R^{44} are each methyl;

R^1 and R^{41} are independently H or sulfonic acid; and

R^6 and R^{46} are H.

27. A compound, as claimed in Claim 24, wherein the compound is substituted one or more times by sulfonic acid.

28. A compound, as claimed in Claim 22, wherein one of R^1 , $R^{1'}$, R^2 , $R^{2'}$, R^3 , R^4 , R^5 , R^6 , R^7 , R^8 , R^{15} , R^{16} , R^{20} , R^{21} , R^{23} , R^{24} , R^{25} , R^{30} , R^{31} , R^{32} , R^{33} , R^{34} , R^{37} , R^{38} , R^{41} , R^{42} , R^{43} , R^{44} , R^{45} , and R^{46} is an $-L-R_x$ or $-L-S_c$.

29. A compound, as claimed in Claim 28, wherein each L is independently a single covalent bond, or L is a covalent linkage having 1-24 nonhydrogen atoms selected from the group consisting of C, N, O, P, and S and is composed of any combination of single, double, triple or aromatic carbon-carbon bonds, carbon-nitrogen bonds, nitrogen-nitrogen bonds, carbon-oxygen bonds, carbon-sulfur bonds, phosphorus-oxygen bonds, and phosphorus-nitrogen bonds.

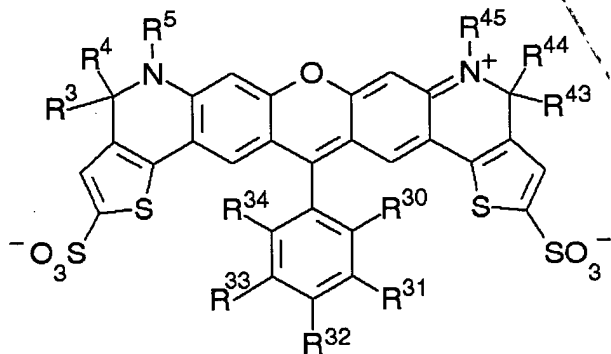
30. A compound, as claimed in Claim 28, wherein R_x is an acrylamide, an activated ester of a carboxylic acid, an acyl azide, an acyl nitrile, an aldehyde, an alkyl halide, an amine, an anhydride, an aniline, an aryl halide, an azide, an aziridine, a boronate, a carboxylic acid, a diazoalkane, a haloacetamide, a halotriazine, a hydrazine, an imido ester, an isocyanate, an isothiocyanate, a maleimide, a phosphoramidite, a reactive platinum complex, a sulfonyl halide, or a thiol group.

31. A compound, as claimed in Claim 28, wherein R_x is a phosphoramidite, a succinimidyl ester of a carboxylic acid, a haloacetamide, a hydrazine, an isothiocyanate, a maleimide group, a perfluorobenzamido, an azidoperfluorobenzamido group, or a reactive platinum complex.

32. A compound, as claimed in Claim 28, wherein S_c is an amino acid, a peptide, a protein, a tyramine, a monosaccharide, a polysaccharide, an ion-complexing moiety, a nucleoside, a nucleotide, an oligonucleotide, a nucleic acid, a hapten, a psoralen, a drug, a hormone, a lipid, a lipid assembly, a polymer, a polymeric microparticle, a biological cell, or a virus.

33. A compound, as claimed in Claim 28, wherein S_c is an amino acid, a peptide, a protein, an ion-complexing moiety, a nucleoside, a nucleotide, an oligonucleotide, or a nucleic acid.

34. A compound, as claimed in Claim 28, having the formula:



wherein R^3 , R^4 , R^5 , R^{43} , R^{44} , and R^{45} are independently methyl or ethyl;

R^{30} is sulfonic acid or carboxylic acid;

R^{31} and R^{34} are independently H, F, or Cl;

one of R^{32} and R^{33} is H, F, or Cl, and the other of R^{32} and R^{33} is $-L-R_x$ or $-L-S_c$,

wherein L is a covalent linkage of the formula $-S(CH_2)_aCOO(CH_2)_b-$ or the formula $-S(CH_2)_aCONH(CH_2)_b-$

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wherein a is an integer between 0 and 10, and b is an integer between 0 and 10 provided that a and b are not both 0; and

wherein R_x, where present, is a carboxylic acid, an activated ester of a carboxylic acid, a haloacetamide, a hydrazine, an isothiocyanate, a maleimide group, or a reactive platinum complex.; and

wherein S_c, where present, is an amino acid, a peptide, a protein, an ion-complexing moiety, a nucleoside, a nucleotide, an oligonucleotide, or a nucleic acid.

10 35. A compound, as claimed in Claim 34, wherein R_x is a maleimide group or is a succinimidyl ester of a carboxylic acid.

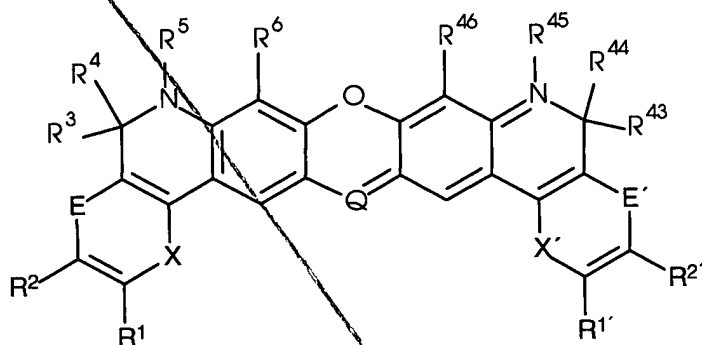
36. A compound, as claimed in Claim 34, wherein S_c is peptide or a protein or a lectin.

15 37. A compound, as claimed in Claim 34, wherein S_c is an antibody or antibody fragment.

38. A compound, as claimed in Claim 34, wherein S_c is a nucleotide or an oligonucleotide.

20 39. A compound, as claimed in Claim 34, wherein S_c is a BAPTA or APTRA ion-complexing moiety.

40. A method of staining a biological sample, comprising:
combining a dye solution comprising a compound of the formula



wherein

10 $R^1, R^2, R^6, R^{41}, R^{42}$, and R^{46} are independently H, cyano, nitro, halogen, carboxylic acid, or sulfonic acid; or a C_1-C_6 alkyl or alkoxy that is optionally substituted by carboxylic acid, sulfonic acid, or halogen; or an aromatic or heteroaromatic ring that is optionally substituted one or more times by C_1-C_6 alkyl, C_1-C_6 perfluoroalkyl, cyano, halogen, azido, carboxylic acid, sulfonic acid, or halomethyl; or $-L-R_x$; or $-L-S_C$;

15 or R^1 in combination with R^2 , or R^{41} in combination with R^{42} , or both, forms a fused aromatic or heteroaromatic ring that is optionally sulfonated one or more times;

20 R^3, R^4, R^{43} , and R^{44} are independently H, C_1-C_6 alkyl that is optionally substituted by carboxylic acid, sulfonic acid, amino, hydroxy, or halogen; or an aromatic or heteroaromatic ring that is optionally substituted one or more times by C_1-C_6 alkyl, C_1-C_6 perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl;

25 or R^2 in combination with R^3 , R^{42} in combination with R^{43} , or R^3 in combination with R^4 , or R^{43} in combination with R^{44} , or any combination thereof, forms a 5- or 6-membered alicyclic ring;

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5 R^5 and R^{45} are independently H, methyl, carboxymethyl, a C_2-C_6 alkyl that is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen; or R^5 is an aryl or heteroaryl ring that is optionally substituted one or more times by C_1-C_6 alkyl, C_1-C_6 perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl;

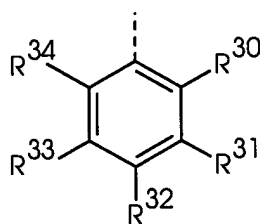
or R^4 in combination with R^5 , or R^5 in combination with R^6 , or R^{44} in combination with R^{45} , or R^{45} in combination with R^{46} , or any combination thereof, forms a 5- or 6-membered alicyclic ring;

one of E and X is O, S, NR^8 , or $CR^{1'}=CR^{2'}$; the other is absent; and one of E' and X' is O, S, NR^8 , or $CR^{1'}=CR^{2'}$; the other is absent;

15 wherein R^8 is H, methyl, carboxymethyl, or a C_2-C_6 alkyl that is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen; and

20 $R^{1'}$ and $R^{2'}$ are independently H, cyano, halogen, carboxylic acid, or sulfonic acid; or a C_1-C_6 alkyl or alkoxy that is optionally substituted by carboxylic acid, sulfonic acid, or halogen; or an aryl or heteroaryl ring that is optionally substituted one or more times by C_1-C_6 alkyl, C_1-C_6 perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl;

25 Q is N or CR^{28} , wherein R^{28} is H, F, CN, carboxylic acid, or a carboxylic acid ester of a C_1-C_6 alcohol; or R^{28} is a C_1-C_6 alkyl that is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen; or R^{28} has the formula



where R^{30} , R^{31} , R^{32} , R^{33} and R^{34} are independently H, F, Cl, Br, I, sulfonic acid, carboxylic acid, CN, nitro, hydroxy, azido, amino, hydrazino; or C_1 - C_{18} alkyl, C_1 - C_{18} alkoxy, C_1 - C_{18} alkylthio, C_1 - C_{18} alkanoylamino, C_1 - C_{18} alkylaminocarbonyl, C_2 - C_{36} dialkylaminocarbonyl, C_1 - C_{18} alkyloxycarbonyl, or C_6 - C_{18} arylcarboxamido, the alkyl or aryl portions of which are optionally substituted one or more times by F, Cl, Br, I, hydroxy, carboxylic acid, a carboxylic acid ester of a C_1 - C_6 alcohol, amino, alkylamino, dialkylamino or alkoxy, the alkyl portions of each having 1-6 carbons; or one pair of adjacent substituents R^{31} and R^{32} , R^{32} and R^{33} or R^{33} and R^{34} , when taken in combination, form a fused 6-membered aromatic ring that is optionally further substituted by carboxylic acid; or one or more of R^{30} , R^{31} , R^{32} , R^{33} and R^{34} is -L- R_x or -L- S_C ; and

wherein L is a covalent linkage;

R_x is a reactive group; and

S_C is a conjugated substance;

with a biological sample in a concentration sufficient to yield a detectable optical response under the desired conditions.

41. A method, as claimed in Claim 40, further comprising combining the sample with an additional detection reagent that has spectral properties that are detectably different from said optical response.

42. A method, as claimed in Claim 40, further comprising the step of determining a characteristic of the sample by comparing the optical response with a standard response parameter.

43. A method, as claimed in Claim 40, wherein the sample comprises cells.

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44. A method, as claimed in Claim 40, wherein the sample is immobilized in or on a solid or semi-solid matrix that is a membrane, an electrophoretic gel, a silicon chip, a glass slide, a microwell plate, or a microfluidic chip.

5 45. A method, as claimed in Claim 40, further comprising tracing the temporal or spatial location of the optical response within the sample.

46. A method, as claimed in Claim 40, wherein for said compound

10 at least one of R^{28} , R^{30} , R^{31} , R^{32} , R^{33} , R^{34} , R^{37} and R^{38} is $-L-R_x$ or $-L-S_c$;

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15 R_x is a carboxylic acid, an activated ester of a carboxylic acid, an amine, an azide, a hydrazine, a haloacetamide, an alkyl halide, an isothiocyanate, or a maleimide group; and

S_c is an amino acid, a peptide, a protein, a polysaccharide, a nucleotide, a nucleoside, an oligonucleotide, a nucleic acid polymer, an ion-complexing moiety, a lipid, or a non-biological organic polymer or polymeric microparticle, that is optionally bound to one or more additional fluorophores that are the same or different.

20 47. A method, as claimed in Claim 46, wherein for said compound, R^{28} is an $-L-S_c$, and S_c is an ion-complexing moiety that is a BAPTA or an APTRA.

25 48. A method as claimed in Claim 40, wherein at least one of R^{28} , R^{30} , R^{31} , R^{32} , R^{33} , R^{34} , R^{37} and R^{38} is $-L-S_c$, and S_c is a nucleoside, a nucleotide, an oligonucleotide, or a nucleic acid polymer.

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